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INVESTIGATION OF
OIL DRILLING IMPACTS TO
AQUATIC HABITAT RESOURCES

IN SITU
BIOLOGICAL ASSESSMENT OF THE
PHOTOINDUCED TOXICITY OF
ENVIRONMENTAL RELEASES OF
CRUDE OIL

by Melanie Y. Young

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U.S. Fish and Wildlife Service
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Abstract

This study proposed a biological assessment of a recent crude oil spill for potential impacts to aquatic resources due to petroleum hydrocarbon wastes. The objectives of the proposed study were to assess the phototoxicity of crude oil releases to the environment (specifically the polycyclic aromatic hydrocarbon fraction) in situ; and to compare the in situ phototoxic response to identical tests conducted under controlled laboratory conditions. Following acute exposure periods, *Lumbriculus variegatus* mortality under natural sunlight at a crude oil contaminated site was significantly higher than corresponding mortality protected from UV radiation. *L. variegatus* experienced greater than 90% mortality in a crude oil contaminated wetland during exposure to sunlight, 0% mortality at reference sites, and less than 40% mortality across corresponding treatments protected from UV radiation. *Pimephales promelas* mortality exposed to sunlight in crude oil contaminated wetlands was not significantly different from corresponding treatments protected from UV radiation. Results indicate that crude oil-contaminated sediments in the aquatic environment have the potential to substantially adversely affect survival of UV-exposed aquatic organisms.

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Introduction

During a recent study conducted by the U.S. Fish and Wildlife Service (Service) of the effects of oil and gas drilling activities on aquatic biological resources (Young 1993), a reduction in benthic organism density was noted in localized areas of crude oil contamination. Polycyclic aromatic hydrocarbons (PAHs) are known to adversely affect some fish species in metabolized forms, and have significant chronic toxicities for various macroinvertebrates. They can accumulate to significantly high proportions in lower trophic level organisms unable to metabolize them, such as aquatic invertebrates. Crude oil is generally composed of saturated hydrocarbons (straight and branched alkanes, and cyloalkanes), aromatic hydrocarbons (aromatics, polycyclic aromatic hydrocarbons, cycloalkanoaromatics, and cyclic sulphur compounds), and resins and asphaltenes (including high molecular weight polycyclic fractions containing nitrogen, sulphur and oxygen compounds) (Gill and Robotham 1989). Although composed primarily of saturated hydrocarbons (57.2%), some crude oils may contain as much as 28% aromatic hydrocarbons (Tissot and Welte 1984).

Accidental crude oil releases make up a notable portion of total petroleum hydrocarbon releases in southeastern Illinois. Sources of crude oil and crude oil-related contamination to surface water resources in this region include crude oil and waste brine fluids from accidental spills; major accidental resurfacing of oil and brine during secondary and tertiary recovery operations; and drilling fluids temporarily stored in surface impoundments. PAHs are a component of crude oil, and some polycyclic aromatic hydrocarbons are known to be phototoxic in nature. Toxic qualities of parent compounds as well as degradation products are stimulated and enhanced by ultraviolet radiation from natural sunlight. Specific polycyclic aromatic hydrocarbons, as well as sediments generally characterized by PAH contamination, have been tested in laboratory bioassays with both invertebrates and vertebrates under conditions simulating natural sunlight, and have demonstrated this effect (Oris and Giesy 1986; Landrum et al. 1987; Davenport and Spacie 1991).

In 1993 the Service conducted comparative laboratory toxicity tests under normal laboratory fluorescent lighting and near ultraviolet lighting conditions using crude oil-contaminated sediments collected from Gallatin County in southern Illinois from an oil pipeline oil spill. Our results indicated significantly different survival using Ceriodaphnia dubia and Hyallela azteca as test organisms. We observed complete mortality of test organisms for the Ceriodaphnia near ultraviolet exposures, and no mortality with the same sediments exposed to fluorescent lighting for the test duration (unpublished data). During an investigation of macroinvertebrate diversity after a recent crude oil spill that occurred in December of 1988 on the lower Gasconade River in central Missouri, researchers at the National Biological Survey, National Fisheries Contaminants Research Center in Columbia, Missouri found a significant reduction in benthic diversity in backwater habitats for a period of time extending to 18 months after the spill had occurred (Barry Poulton, pers. comm.). What was discovered during the course of the assessment was that after the release, oil had accumulated in backwater habitats and had become incorporated into the bottom sediments (which apparently had not been remediated). It is worthwhile to note also that the initial biological assessment of this spill did not even begin until after approximately three months after the spill had originally occurred, another indication of the potential long-term adverse effects of only partially remediated spills.

Other researchers are just beginning to utilize in situ monitoring techniques with Ceriodaphnia to assess PAH contamination in stormwater, and have identified the significance of phototoxic effects in the natural environment (Ireland et al. 1993).

The objectives of the proposed study were to assess the phototoxicity of a major crude oil release to the environment (specifically the polycyclic aromatic hydrocarbon fraction) in situ; and to compare the in situ phototoxic response to similar tests conducted under controlled laboratory conditions. Resource management personnel may utilize the data obtained in the present study to assess the severity of impacts to aquatic habitat resources following a crude oil release. This data may also be useful in land acquisition proceedings involving potential acquisition of lands affected by oil and gas drilling or other petroleum-related industry.

Study Area Description

The study area was the site of a recent pipeline rupture in Knox County, Illinois In August of 1993 a 12" crude oil pipeline owned by Amoco Oil Company was accidentally struck by a local service truck and ruptured, spilling approximately 42,000 gallons of crude oil into Brush Creek. Brush Creek empties into Lake Bracken approximately 3/4 miles downstream, and although attempts were made to contain the oil adjacent to the spill site, a substantial quantity had escaped and entered Lake Bracken (Figure 1). Oil-soaked birds and other wildlife were reported, as well as fish kills. Lake Bracken is a 300 acre recreational lake located approximately 3 miles south of Galesburg, Illinois. Brush Creek enters into an alluvial fan at the western edge of Lake Bracken, which is where much of the oil accumulated after booms were placed at this end to prevent further spread of oil throughout the remainder of Lake Bracken. The alluvial fan at the western edge of Lake Bracken, comprised of open water habitat with emergent vegetation, was the area in which the in situ tests were set up.

Methodology

In Situ Toxicity Tests

One site was selected adjacent to booms placed in the western edge of Lake Bracken for in situ field analysis. *Lumbriculus variegatus*, and *Pimephales promelas* (larvae) were utilized in the in situ exposures. Two groups of four in situ chambers (2" glass cylinders) per species (one group of four for *P. promelas*) were placed at the site. An additional site was selected as a reference location from a nearby uncontaminated area for comparative purposes. Chambers were placed in areas at both experimental and reference sites, and half were exposed and half protected from ultraviolet radiation. After initial pre-exposures, the tests were conducted for approximately 4-8 hours, upon exposure to ultraviolet radiation from natural sunlight. The tests were terminated after complete mortality of one or more test replicates for either species, and before 20% mortality of control replicates. The test parameters measured for field tests will be organism counts at the start and end of ultraviolet exposures; organism mortality on a bi-hourly basis after the start of the test; and water quality parameters (temperature, pH, DO, conductivity, salinity, depth, and turbidity). Temperature, dissolved oxygen, pH, conductivity, and turbidity were measured with a Solomat multi-functional water quality monitor, Model 520c. Salinity was measured

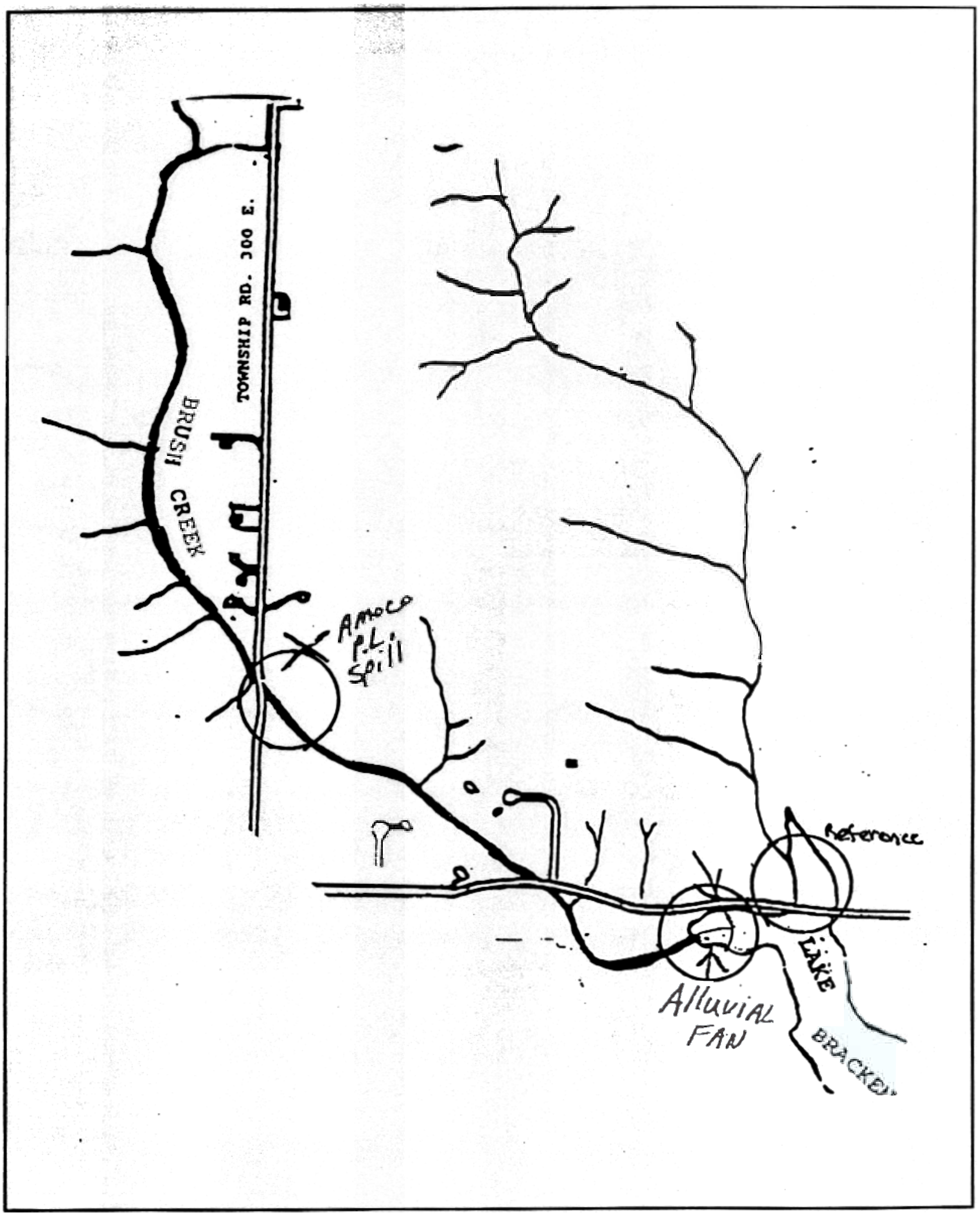

Figure 1. Lake Bracken study area test sites.

with a YSI Model 33 S-C-T meter. Depth was measured with a depth tape. All meters were calibrated to manufacturers' specifications prior to use. Tests were conducted and samples were collected and processed according to established protocols (American Society for Testing and Materials 1988, 1991; Nebeker et al 1984).

Laboratory Toxicity Tests

A toxicity testing laboratory was contracted to conduct comparative laboratory exposures with sediment samples collected from the study site. Laboratory toxicity testing was conducted at Purdue University, Lafayette, Indiana. All tests were conducted using neonate *Ceriodaphnia dubia*, in acute, static porewater exposures.

A composite surficial bottom sediment sample was collected from the Lake Bracken study site, and the field reference location. An additional laboratory reference sediment sample was incorporated into the testing parameters; porewater was prepared from sediment samples collected from unpolluted Taylor Lake in Upland, Indiana. This material has been used previously by the contracted toxicity testing facility as a control in porewater experiments, and has not shown toxicity to *C. dubia*. All samples were collected according to standard protocols (ASTM 1991). Sediments were collected with decontaminated stainless steel utensils. Each sample was thoroughly mixed, then proportioned into 1000 ml chemically cleaned amber borosilicate glass jars with Teflon™-lined lids. All sample containers were shielded from accidental sunlight exposure prior to test initiation. Sampling equipment was rinsed and decontaminated between the crude oil spill site and reference sampling locations. Sample containers were labeled and temporarily stored at 4°C in the field, and then shipped the same day of collection overnight to the toxicity testing facility.

Sediment samples were stored at 4°C upon receipt, and held for three days prior to preparation of porewater. For each of the bulk sediment samples, 8 subsamples were placed in wide-mouth high density polyethylene centrifuge bottles and centrifuged at 5000 rpm for 30 minutes. The extracted porewaters were then decanted and filtered through a glass fiber filter to remove particulates. The Lake Bracken and reference sediment porewater samples were then aerated prior to test initiation to raise dissolved oxygen levels to the acceptable standard of >5ppm for static toxicity tests, according to standard protocols (ASTM 1988), and subsequently used immediately for toxicity testing.

The experimental design called for preparation and testing of porewater from crude oil and reference sediment samples collected, concurrently with organism culture water controls, and anthracene controls. The test parameters measured for laboratory tests were organism counts at the start and end of ultraviolet exposures; organism mortality on a bi-hourly basis after the start of the test; and water quality parameters (temperature, pH, DO, hardness, alkalinity, and conductivity). Tests were conducted according to established protocols (American Society for Testing and Materials 1988, 1991; Nebeker et al. 1984).

To assess organism health, to provide a minimal physiological uptake period for potentially phototoxic PAHs, and to control for toxicity not induced by the light regimes, approximately ten organisms (four replicates) were pre-exposed to crude oil and reference sediment porewater, water control, and anthracene control test solutions for 12 hours in total darkness at 23.0°C. All organisms were inspected at the beginning and end of the pre-exposure period. All tests were performed in 30 ml polystyrene cups. Aliquots of each test solution (15 ml each) were distributed to the 4 replicate chambers. Approximately ten neonates were then added under the water surface of each chamber. Cup position was randomized; the cups were covered with a plexiglas sheet, and the entire rack was held in the dark for 12 hours at 24° C. This constituted the physiological uptake phase of the test, after which each cup was counted to determine initial survival. Any significant mortality after 12 hours would indicate conventional, or "dark" toxicity of the solution.

A known phototoxicant (Oris and Giesy 1987), anthracene, was used as a control in the tests for comparison with the phototoxic response of PAHs in crude oil. Anthracene (M.W. 178.23) is a linear three-ring polycyclic aromatic hydrocarbon (Oris and Giesy 1987), and exhibits median levels of photo-induced acute toxicity to aquatic organisms relative to other PAHs (Neff 1979). Also, the effects of anthracene on *C. dubia* have been well-studied. A stock solution of anthracene in methanol (4000 μg/L) was prepared, and then a series of anthracene dilutions were prepared in culture water with the following nominal concentrations: 2, 4, 8, and 16 μg/L. The 4

$\mu g/L$ concentration, which was the minimum concentration to demonstrate complete toxicity to test organisms, was included in the testing design with the porewater treatment and control samples. Methanol was included in the control treatment (data not shown). Extensive testing with methanol at the contracted toxicity testing facility has shown that it produces no toxicity or synergistic effects when used in acute phototoxicity tests with *C. dubia*. Methanol alone demonstrated no toxicity to test organisms over this same exposure period.

Following the 12 hour uptake phase, test chambers were randomly distributed and then held under either cool white fluorescent (representing normal laboratory lighting) or near-UV illumination. Lamps used were 20 watt Westinghouse cool white fluorescent bulbs (without measurable UV radiation), or 20 watt GE BLB black lights (max UV-A wavelength 360 nm). BLB lamps produce virtually the same UV-A intensity at 355 nm as Q-panel lamps that are designed to simulate the natural UV spectrum. Chambers were held at a distance of 70 mm from the lamps. The UV intensity, measured with a IL500 light meter, was 1300 $\mu watts/cm^2$ at the surface, and 1267 $\mu watts/cm^2$ at the bottom of the chambers. By comparison, ambient daylight UV-A intensity ranges from approx. 1600 $\mu watts/cm^2$ in January, when the sky is fully overcast, to 3000 $\mu watts/cm^2$ in June, in the northern United States. Such levels have been shown to be harmless to *C. dubia* during short-term exposures.

All tests were conducted using neonate *C. dubia*, <24 h old, from a stock that has been cultured continuously in the laboratory for approximately 10 years. Stock cultures in brood boards were maintained in an incubator at $25 \pm 1.0°$ C on a 16 hours light / 8 hours darkness cycle under cool white fluorescent lights. Water for all stock cultures and test dilutions was moderately hard U.S. Environmental Protection Agency formula reconstituted water (pH 7.4-7.8; hardness 80 - 100 mg/L; alkalinity 60-70 mg/L).

Toxicity tests were initiated upon exposure of test organisms to near-UV and fluorescent light regimes. Test chamber temperature was monitored bi-hourly throughout the exposure period; chambers were checked and mortalities counted every two hours. Tests were terminated after complete mortality in the crude oil sediment porewater treatment replicates (under near-UV light) occurred, and prior to a maximum of 20% mortality in any control replicates. All toxicity testing was initiated and completed within one week of initial sample collections. Complete immobilization was used as the mortality criterion in this test.

Chemical Analysis

Surficial bed sediment composite samples were collected at the study and reference sites for chemical/physical analyses. Samples were collected according to standard protocols (ASTM 1991). Sediments were collected with decontaminated stainless steel utensils. Each sample was thoroughly mixed, then proportioned into 1000ml chemically cleaned amber borosilicate glass jars with Teflon™-lined lids. All sample containers were shielded from accidental sunlight exposure prior to analysis. Sampling equipment was rinsed and decontaminated between the crude oil spill site and reference sampling locations. Sample containers were labeled and temporarily stored at 4°C in the field, and then shipped under dry ice shortly after collection overnight to the analytical facility for chemical analysis. Bed sediment samples were analyzed for polycyclic aromatic hydrocarbons via capillary gas chromatography. Grain size analysis, percent moisture, and total organic carbon content were made on separate bed sediment subsamples. Chemical analyses was conducted by Geochemical and Environmental Research Group, Texas A & M University, College Station, Texas.

For grain size determinations, a small aliquot of sediment was treated with 30% hydrogen peroxide to remove organic coating from grains. A dispersing agent was then added to the sample. The sand/mud fractions were then separated using a 63 micron sieve. The sand fraction (>63 microns) was retained on the screen and the mud fraction (silt and clay <63 microns) was washed into a 1 (one) liter volumetric cylinder. The sand fraction was dried, sieved on a 63 micron screen and weighed. The sediment which passes through the screen a second time was added to the 1 liter cylinder. The mud fraction was analyzed by stirring the cylinder and sampling 20 ml aliquots at 4 and 8 phi intervals. The 4 and 8 phi samples were dried and weighed. The percent sand, silt, and clay fractions were determined on a dry weight basis.

For the percent moisture determination, approximately 1 gram of wet sample was weighed into a clean, labeled, preweighed 10 ml beaker. The beaker was placed in a forced air oven at approximately 75 degrees Celsius for 24 hours. The beaker with the dry sample was then weighed and the % dry weight calculated from that aliquot.

For the total organic carbon determinations, the Leco method was utilized which involves burning an acidified freeze dried sediment sample using a LECO Model 523-300 induction furnace under an oxygen environment. The resultant carbon dioxide was detected and quantified with a Horiba PIR-2000 infrared detector. The output signal from the Horiba is sent to an HP 3396A integrator which reports the quantity of carbon dioxide as a peak area.

For the aromatic/aliphatic analyses, the sediment samples were freeze-dried and extracted in a Soxhlet extraction apparatus. Briefly, the freeze-dried sediment samples were homogenized and a 10-gram sample was weighed into the extraction thimble. Surrogate standards and methylene chloride were added and the samples extracted for 12 hrs. The extracts were treated with copper to remove sulfur and were purified by silica/alumina column chromatography to isolate the aliphatic and aromatic/pesticide/PCB fractions. The quantitative analyses were performed by capillary gas chromatography (CGC) with a flame ionization detector for aliphatic hydrocarbons, and a mass spectrometer detector in the SIM mode for aromatic hydrocarbons.

The limits of detection were approximately 0.001 (mg/kg dry weight) (varied by sample). For the organic analysis of sediments, the accuracy and precision of the chemical analysis was measured by reagent and matrix blank sample analysis, spike recovery sample analysis, and duplicate sample analysis, with confirmation of selected samples by gas chromatography/ mass spectrometry by the analytical facility. Quality assurance and quality control for sediment sample analyses was monitored by the U.S. Fish and Wildlife Service Patuxent Analytical Control Facility, Laurel, Maryland, and was reported as acceptable for all analytes.

Results

L. variegatus mortality was significantly higher (Chi-square; $p=0.001$) between treatments under sunlight exposed and UV-protected exposures (**Table 1(a)**). *L. variegatus* experienced no mortality across treatments for both reference samples for the entire exposure period. Under UV light at the end of the exposure period, 100% mortality of *L. variegatus* was observed in the crude oil sediment exposures, while 0% mortality was observed across treatments protected from UV light exposure.

P. promelas mortality was also significantly higher (Chi-square; $p=0.001$) between treatments under sunlight exposed and UV-protected exposures (**Table 1(b)**. *P. promelas* experienced slight mortality across treatments for both reference samples for the entire exposure period. Under UV light at the end of the exposure period, 55% mortality of *P. promelas* was observed in the crude oil sediment exposures, while 12% mortality was observed across treatments protected from UV light exposure.

For the controlled laboratory exposures, it appears that *C. dubia* mortality also approaches significance between treatments under UV-exposed and UV-protected exposures (**Table 1(c)**.

Discussion

The results indicate that *L. variegatus* was extremely sensitive to PAHs in crude oil-contaminated sediments under natural sunlight (UV light) exposure, and suggest that the exposure to UV radiation caused the phototoxic response. The reference sediment sample demonstrated no substantial toxicity to *L. variegatus* under either exposure regimes (**Tables 2-7**).

The insignificant mortality of *P. promelas* with crude oil and reference sediment suggests that this organism may be exhibiting less sensitivity to photoactivated PAHs.

Previous gas chromatography/high performance liquid chromatography PAH analysis of crude oil samples and concurrently collected crude oil-contaminated sediments from the southeastern Illinois region, indicated only the presence of phenanthrene in crude oil (as well as in crude oil-contaminated sediments) from this locale (Young 1993).

TABLE 1(a). General Summary - In situ sunlight and UV protected exposures - *Lumbriculus variegatus*.

Lumbriculus variegatus		
	Perecnt Mortality	
	Sunlight Exposed	UV Protected
Test Groups		
Group A		
Composite 1	95	22
Composite 2	100	37
Group B		
Composite 1	90	32
Composite 2	97	40
Reference	0	0

TABLE 1(b). General Summary - In situ sunlight and UV protected exposures - *Pimephales promelas*.

Pimehales promelas		
	Perecnt Mortality	
	Sunlight Exposed	UV Protected
Test Groups		
Group A		
Composites 1/2	42	12
Group B		
Composites 1/2	55	12
Reference 1/2	7	0

TABLE 1(c). General Summary - Laboratory exposures - *Ceriodaphnia dubia.*

Ceriodaphnia dubia		
	Perecnt Mortality	
	UV Exposed	UV Protected
Test Groups		
Lake Bracken	20	7
Field Reference	2	5
Lab Reference	0	2

Additional gas chromatography/mass spectrometry analysis of the crude oil-contaminated sediment samples collected for the present study also revealed the presence of phenanthrene, out of a scan for 19 PAHs (Table 8). Phenanthrene is a three-ring low molecular weight PAH, which has a water solubility of 1.18 mg/L (at 25°C) (U.S. Environmental Protection Agency 1993). Although phenanthrene is not anticipated to exhibit photo-induced toxicity (Newsted and Giesy 1987; Oris and Giesy 1987), the present data seems to suggest that it may at least be partly responsible for eliciting the phototoxic response for *L. variegatus* in this experiment. Phenanthrene has also been previously demonstrated to be phototoxic to aquatic plants (Huang *et al.* 1993).

This project demonstrates that the photo-induced toxicity of PAHs in crude oil may have a potentially significant adverse effect on indigenous biota in an aquatic community, in environments exposed to UV radiation. The results also suggest that remedial management of crude oil-contaminated sediments following major spill events should seriously take into consideration the potential for chronic adverse effects of un-remediated sediments in an aquatic community. With respect to general management implications of lands contaminated by crude oil spills, acquisition of such lands should be handled with caution wherever possible due to the potential acute toxicity of environmental media, as well as the potential difficulty in physically remediating or removing such contaminants from aquatic environments.

This study also has management implications for spill response activities. Major oil spills have previously been documented in the State of Illinois (Osborne et al. 1992). With the extensive oil and gas industry in the state, coupled with the frequency of occurrence of oil spills, such spills need to be assessed in terms of the potential for long-term adverse impacts to benthos due to the phototoxic effects of PAHs remaining in the sediments after initial (if any) spill remediation. The Oil Pollution Act of 1990 (OPA) requires government and oil industry representatives to respond to and remediate oil spills that may occur, as well as to develop contingency plans for removing oil from the environment in the event of a petroleum product spill. Additional proposed regulations (National Atmospheric and Oceanic Administration 1994) regarding the assessment of natural resources damages resulting from such spills will require the assessment of damages to natural resources following a spill. Such requirements necessitate that site remediation measures are adequate to preclude subsequent long-term as well as additional short-term adverse impacts to the environment, particularly the biotic component. Remediation of sites with a history of oil spills should be carried out to the extent that long-term adverse impacts are averted, and, in response to the newly proposed regulations, such remediation measures may need to be more extensive than previously and currently directed.

The present study assessed the phototoxicity of a major crude oil release to the environment (specifically the polycyclic aromatic hydrocarbon fraction) in situ; and to compared the in situ phototoxic response to similar tests conducted under controlled laboratory conditions. Resource managers may use the data obtained in the present study to assess the severity of impacts to aquatic habitat resources following a crude oil release. These data may also be useful in land acquisition proceedings involving potential acquisition of lands affected by oil and gas drilling or other petroleum-related industry.

Conclusions

1. The differences in mortality that occurred in *L. variegatus* during in situ exposure to crude oil sediments between exposed to and protected from UV radiation suggests that organism mortality is due to a phototoxic response.

2. Although *P. promelas* test results were not as definitive as the *L. variegatus* results, the difference in mortality observed between exposed and protected UV exposures at the study site suggests that *P. promelas* was also responding to a greater sensitivity to PAHs under natural sunlight.

3. Environmental exposure of aquatic organisms to UV radiation should be taken into consideration when extrapolating PAH toxicity test laboratory data to effects on field organisms, particularly when such tests are conducted under simulated or actual near-UV exposure conditions. Current laboratory toxicity testing conducted under white fluorescent light may underestimate the degree of toxicity of some PAHs to aquatic biota in natural communities.

4. Remedial management of crude oil-contaminated sediments following major spill events should seriously take into consideration the potential for chronic adverse effects of un-remediated sediments in an aquatic community, and acquisition of lands impacted by oil spills should be avoided wherever possible by resource management officials due to toxicity and difficulties in physically removing such contaminants from aquatic environments.

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Appendix **A**

Organism Sunlight (UV) Exposure Mortality Data

TABLE 2. *Lumbriculus variegatus* bihourly mortality totals and percent mortality for sunlight exposed replicates at experimental and reference sites.

Lumbriculus variegatus				
Sunlight Exposure				
Test Groups (n)	**Total Mortality**			**Percent Mortality[a]**
	0hr	**2hr**	**4hr**	
Group A				
Composite 1 (40)	**0**	**20**	**38[b]**	**95**
Composite 2 (40)	**0**	**18**	**40**	**100**
Group B				
Composite 1 (40)	**0**	**19**	**36**	**90**
Composite 2 (40)	**0**	**25**	**39**	**97**
Reference (40)	**0**	**0**	**0[c]**	**0**

[a]Percent mortality at the test termination at 4hr

[b]Escaped organisms resulted in less than 40/composite at test termination

[c]Two escapees resulted in less than 40/composite at test termination; percent mortality based on number present at test termination

TABLE 3. *Pimephales promelas* bihourly mortality totals and percent mortality for sunlight exposed replicates at experimental and reference sites.

Pimephales promelas						
Sunlight Exposure						
Test Groups (n)	Total Mortality					Percent Mortality*
	0hr	2hr	4hr	6hr	8hr	
Group A 1) 20	0	0	2	6	9	42
2) 20	0	0	1	5	8	
Group B 1) 20	0	0	2	8	12	55
2) 20	0	0	2	7	10	
Reference 1) 20	0	0	0	1	2	7
2) 20	0	0	0	0	1	

Percent mortality at the test termination at 8hr

Appendix B

Organism UV Protected Exposure Mortality Data

TABLE 4. *Lumbriculus variegatus* bihourly mortality totals and percent mortality for UV protected replicates at experimental and reference sites.

Lumbriculus variegatus				
UV Protected				
Test Groups (n)	Total Mortality			Percent Mortality[a]
	0hr	2hr	4hr	
Group A				
Composite 1 (40)	0	0	9[b]	22
Composite 2 (40)	0	7	15	37
Group B				
Composite 1 (40)	0	3	13	32
Composite 2 (40)	0	4	16	40
Reference (40)	0	0	0[c]	0

[a]Percent mortality at the test termination at 4hr

[b]Escaped organisms resulted in less than 40/composite at test termination

[c]Two escapees resulted in less than 40/composite at test termination; percent mortality based on number present at test termination

TABLE 5. *Pimephales promelas* bihourly mortality totals and percent mortality for UV protected replicates at experimental and reference sites.

Pimephales promelas						
UV Protected						
Test Groups (n)	Total Mortality					Percent Mortality[a]
	0hr	2hr	4hr	6hr	8hr	
Group A 1) 20	0	0	0	1	3	12
2) 20	0	0	0	1	2	
Group B 1) 20	0	0	0	1	2	12
2) 20	0	0	0	3	3	
Reference 1) 20	0	0	0	0	0	0
2) 20	0	0	0	0	0	

Percent mortality at the test termination at 8hr

Appendix C

Site W ter Quality Data

TABLE 6(a). Water quality parameter measurements for Lake Bracken at initiation (hour 0) and termination (hour 8).

Water Quality Parameters		
Lake Bracken		
	Initiation (hour 0)	Termination (hour 8)
Parameter		
DO (ppm)	8.62	8.19
pH (H^+)	4.4	4.54
Temperature (°C)	24	29
Salinity (%)	0	0
Depth (inches)	8	8
Conductivity ($\mu S/cm^3$)	720	800
Turbidity (NTU)	140	140

TABLE 6(b). Water quality parameter measurements for reference site at initiation (hour 0) and termination (hour 8).

Water Quality Parameters		
Reference Site		
	Initiation (hour 0)	Termination (hour 8)
Parameter		
DO (ppm)	5.08	6.00
pH (H^+)	4.23	4.58
Temperature (°C)	25	28
Salinity (%)	0	0
Depth (inches)	8	8
Conductivity ($\mu S/cm^3$)	360	380
Turbidity (NTU)	40	41

Appendix D

Laboratory Phototoxicity Test Data

TABLE 7(a). *Ceriodaphnia dubia* bihourly laboratory test mortality totals and percent mortality for UV exposed replicates for experimental and reference site samples.

Ceriodaphnia dubia							
UV Exposed							
Test Groups (n)	Total Mortality						Percent Mortality[a]
	0hr	2hr	4hr	6hr	8hr	10hr	
Lake Bracken Sediment (41)	0	0	1	3	6	8	20
Field Reference Sediment (41)	0	0	0	0	1	1	2
Anthracene Control (39)	0	13	34	39	-	-	100
Water Control (41)	0	0	0	0	0	1	2
Laboratory Reference Sediment (39)	0	0	0	0	0	0	0

[a]Percent mortality at the test termination

TABLE 7(b). *Ceriodaphnia dubia* bihourly laboratory test mortality totals and percent mortality for UV protected replicates for experimental and reference site samples.

Ceriodaphnia dubia							
UV Protected							
Test Groups (n)	Total Mortality						Percent Mortality[a]
	0hr	2hr	4hr	6hr	8hr	10hr	
Lake Bracken Sediment (42)	0	0	1	1	3	3	7
Field Reference Sediment (40)	1	1	1	1	2	2	5
Anthracene Control (41)	1	1	1	1	1	1	2
Water Control Not Conducted							
Laboratory Reference Sediment (43)	0	0	0	1	1	1	2

[a]Percent mortality at the test termination

TABLE 7(c). Water quality parameter measurements for laboratory phototoxicity tests at initiation (hour 0) and termination (hour 10).

Water Quality Parameters				
Laboratory Phototoxicity Tests				
	Initiation (hour 0)		Termination (hour 8)	
Parameter	Lake Bracken	Field Reference	Lake Bracken	Field Reference
DO (ppm)	8.9	7.8	6.8	6.2
pH (H^+)	6.9	6.7	7.2	7.5
Temperature (°C)	24.5	24.2	24.9	24.8
Hardness (mg/L)	255	280	255	275
Alkalinity (mg/L)	165	180	160	180
Conductivity ($\mu S/cm^3$)	480	435	490	430

Appendix E

Analytical Chemistry Data: Bottom Sediments

Table 8. Concentrations (mg/kg dry weight) of aromatic and aliphatic hydrocarbons detected in sediments collected from Lake Bracken in 1995.

Analyte	Sample Location		
	Lake Bracken	Brush Creek	L. Bracken Reference
% Moisture	54.6	32.47	57.77
% Total Organic Carbon	4.03	1.69	3.06
% Sand	5.37	34.88	5.73
% Silt	51.53	46.55	55.35
% Clay	43.1	18.57	38.92
1,2,5,6-dibenzanthracene	0.0101	0.0034	0.0011
1,2-benzanthracene	0.1136	0.0156	0.0035
1,6,7-Trimethyl-naphthalene	0.6321	0.1498	0.0049
1-methylnaphthalene	0.5746	0.0568	0.0011
1-methylphenanthrene	0.2295	0.0427	0.0011
2,6-dimethylnaphthalene	1.0426	0.1538	0.0068
2-methylnaphthalene	0.6823	0.0497	0.0021
Cl-Fluoranthenes & Pyrenes	0.3162	0.0485	0.0104
Cl-Phenanthrenes & Anthracenes	0.8189	0.1735	0.0054
Cl-chrysenes	0.2788	0.0629	0.0068
Cl-dibenzothiophenes	0.3259	0.0691	<0.00
Cl-fluorenes	0.3638	0.0773	0.0030
Cl-naphthalenes	1.2569	0.1064	0.0033
C2-Phenanthrenes & Anthracenes	1.1743	0.2609	0.1491
C2-chrysenes	0.3006	0.0700	<0.00
C2-dibenzothiophenes	0.6257	0.1392	0.0042
C2-fluorenes	0.7371	0.1691	0.0071
C2-naphthalenes	1.7248	0.3426	0.0149
C3-Phenanthrenes & Anthracenes	1.1983	0.2702	0.0191
C3-chrysenes	0.0281	0.0116	<0.00
C3-dibenzothiophenes	0.5759	0.1263	0.0071
C3-fluorenes	0.7717	0.1867	0.0028

Table 8 (cont.). Concentrations (mg/kg dry weight) of aromatic and aliphatic hydrocarbons detected in sediments collected from Lake Bracken in 1995.

Analyte	Sample Location		
	Lake Bracken	Brush Creek	L. Bracken Reference
C3-naphthalenes	1.8459	0.5791	0.0168
C4-Phenanthrenes & Anthracenes	0.4008	0.0989	0.0071
C4-chrysenes	0.0169	0.0065	<0.00
C4-naphthalenes	1.2598	0.4629	<0.00
acenaphthalene	0.0266	0.0053	0.0014
acenaphthene	0.0259	0.0056	<0.00
anthracene	0.0940	0.0171	0.0021
benzo(a)pyrene	0.0537	0.0088	0.0028
benzo(b)fluoranthene	0.1504	0.0220	0.0071
benzo(e)pyrene	0.0905	0.0193	0.0037
benzo(g,h,i)perylene	0.0288	0.0118	0.0097
benzo(k)fluoranthene	0.0279	0.0057	0.0016
biphenyl	0.1154	0.0168	<0.00
chrysene	0.2222	0.0482	0.0061
dibenzothiophene	0.1374	0.0256	0.0011
fluoranthene	0.2455	0.0324	0.0113
fluorene	0.1422	0.0256	0.0033
indeno(1,2,3-cd)pyrene	0.0262	0.0096	0.0040
phenanthrene	0.2876	0.0503	0.0044
phenanthrenequinone	<0.02	<0.01	<0.02
n-decane	0.2438	0.0687	<0.00
n-docosane	0.2814	0.3416	0.0483
n-dodecane	0.8400	0.3346	<0.00
n-dotriacontane	0.3786	0.0632	0.1148
n-eicosane	0.2138	0.4449	0.0627
n-heneicosane	0.1165	0.3237	0.1622
n-hentriacontane	<0.00	0.2113	0.6450

Table 8 (cont.). Concentrations (mg/kg dry weight) of aromatic and aliphatic hydrocarbons detected in sediments collected from Lake Bracken in 1995.

Analyte	Sample Location		
	Lake Bracken	Brush Creek	L. Bracken Reference
n-heptacosane	1.9940	0.3485	0.8659
n-heptadecane	1.2295	0.7596	0.2171
n-hexacosane	0.3273	0.1682	0.1283
n-hexadecane	0.8995	0.9506	0.0322
n-nonacosane	6.1312	1.2766	3.9277
n-nonadecane	0.8332	0.5587	0.0944
n-octacosane	0.4482	0.1215	0.2256
n-octadecane	0.2444	0.4900	0.0336
n-pentacosane	1.4361	0.3468	0.4345
n-pentadecane	1.1116	1.0725	0.0201
n-tetracosane	0.1812	0.2276	0.0497
n-tetradecane	0.7321	0.8157	<0.00
n-tetratriacontane	<0.00	0.1572	0.3971
n-triacontane	0.3839	0.1451	0.1065
n-tricosane	0.1555	0.2708	0.1541
n-tridecane	0.5751	0.6158	<0.00
n-tritriacontane	<0.00	0.4895	1.1593
n-undecane	0.4253	0.1505	<0.00
naphthalene	0.1244	0.0148	0.0037
perylene	0.1292	0.0088	0.3596
phytane	9.1301	1.8341	0.0722
pristane	10.584	2.4193	0.0511
pyrene	0.1883	0.0271	0.0075
unresolved complex mixture	1212	225.8	74.82